

The first ptychanthoid Lejeuneaceae in Miocene Mexican amber

Armin Scheben¹, Alexander R. Schmidt², Alfons Schäfer-Verwimp³,
Mónica M. Solórzano Kraemer⁴, Jochen Heinrichs^{1,5}

¹ Systematic Botany and Mycology, Ludwig Maximilian University of Munich, Menzinger Straße 67,
80638 Munich, Germany

² Department of Geobiology, University of Göttingen, Goldschmidtstraße 3, 37077 Göttingen, Germany

³ Mittlere Letten 11, 88634 Herdwangen-Schönach, Germany

⁴ Senckenberg Forschungsinstitut und Naturmuseum, Senckenberganlage 25, 60325 Frankfurt am Main, Germany

⁵ Author for correspondence: jheinrichs@lmu.de

Abstract

Two inclusions of leafy liverworts in Miocene Mexican amber are tentatively assigned to the extant genus *Mastigolejeunea* (Lejeuneaceae subfam. Ptychanthoideae). Both specimens share complicate bilobed leaves with a rounded lobe apex, a lobule with an oblique free margin continuing into the ventral margin of the leaf lobe, elongate leaf cells, a broad ventral merophyte, and undivided underleaves with a truncate to slightly emarginate, recurved apex. The lack of generative structures prevents a more thorough taxonomic treatment. Nevertheless, the inclusions represent the first ptychanthoid Lejeuneaceae from Mexican amber.

Introduction

Fossils are rarely as outstandingly preserved as in amber, fossilized tree resin. For this reason, amber fossils present an invaluable resource for reconstructing palaeoecosystems (Grimaldi 1996; Schmidt et al. 2006, 2010). As with other fossils, they can also be used to calibrate molecular clocks and can thus play an important role in dating evolutionary trees (Donoghue and Benton 2007; Heinrichs et al. 2007; Cooper et al. 2012; Feldberg et al. 2013, 2014; Laenen et al. 2014; Sun et al. 2014). Particularly small, delicate organisms such as arthropods are commonly preserved in amber. Bryophytes, however, despite their small size, are only sparsely represented in the amber fossil record. Indeed, bryophyte inclusions have been found only in nine of the hundreds of known amber deposits, specifically, in Miocene Dominican amber (Gradstein 1993; Frahm and Newton 2005; Heinrichs and Schmidt, 2010; Reiner-Drehwald et al. 2012), Miocene Mexican amber (Grolle 1984; Heinrichs et al. 2014a), Miocene Sicilian amber (Skalski and Veggiani, 1990), Oligocene Bitterfeld and Eocene Baltic amber (Grolle and Meister 2004; Frahm 2004, 2010), Eocene Rovno amber (Ignatov and Perkovsky 2011; Konstantinova et al. 2012), hitherto not precisely dated, presumably Miocene Australian Cape York amber (Hand et al. 2010), Cenomanian-Turonian Alaskan amber (Heinrichs et al. 2011), and Albian-Cenomanian Burmese amber (Heinrichs et al. 2012; Hedenäs et al. 2014; Heinrichs et al. 2014b).

Of the above deposits, Mexican amber has received relatively little attention from researchers, especially in respect to bryophytes, and only two liverworts from this deposit – *Lejeunea palaeomexicana* Grolle and *Ceratolejeunea antiqua* Heinrichs and Schäf.-Verw. (Lejeuneaceae subfam. Lejeuneoideae, Grolle 1984; Heinrichs et al. 2014a) – have been described, so far. To the best knowledge of the authors, no mosses have yet been recognized. Yet Mexican amber warrants study, particularly because it derives from the same angiospermous source trees as the coeval and more thoroughly investigated Dominican amber (early to middle Miocene, 15–20 Mya; Solórzano Kraemer, 2007, 2010; Iturralde-Vinent and MacPhee 1996). Amber from both deposits is attributed to the Fabaceae genus *Hymenaea* (Poinar 1991; Langenheim 1995; Poinar and Brown 2002) and not to coniferous trees like most other ambers.

The fossils described here represent the first ptychanthoid Lejeuneaceae to be described from Mexican amber.

Materials and methods

Both studied amber pieces are part of the Mexican amber collection of the Stuttgart State Museum of Natural History (coll. no. SMNS Mx 352 and SMNS Mx 357). They were ground and polished manually with a series of wet silicon carbide abrasive papers [grit from FEPA P 600–4000 (25.8 µm to 5 µm particle size), firm Struers] to minimize light scattering during the investigation. All inclusions were investigated using a compound microscope (Carl Zeiss AxioScope A1) equipped with a Canon 60D digital camera. In most cases, incident and transmitted light were used simultaneously. The images of Figures 1 and 2 are digitally stacked photomicrographic composites of 23 to 62 individual focal planes obtained using the software package HeliconFocus 5.0 for a better illustration of the three-dimensional inclusions.

Results and Discussion

Description. Upper parts of two sterile, unbranched, dark brownish gametophytes, 2.3 and 2.7 mm long respectively and 0.70–0.95 mm wide. Stem straight, 75–110 µm in diameter; ventral merophyte c. 5 cells wide. Leaves imbricate, incubously inserted, convex, lobes ovate, 0.6–0.9 × 0.3–0.4 mm, apex rounded, plane, margins entire, ventral margin recurved; median cells elongate-hexagonal, 15–25(–35) × 10–15(–20) µm, trigones subnodulose, intermediate thickenings occasionally present on long cell walls, partly subconfluent; lamina cells often collapsed, leaves thus may be broader in natural state; oil bodies not observed. Lobules ovate-rectangular, 0.3–0.4 × 0.1 mm, $2/5$ – $1/2$ times the leaf lobe length, free margin incurved, apical margin not visible, apex continuing into the ventral leaf margin. Underleaves imbricate, undivided, slightly squarrose, c. 0.2–0.3 mm long × 0.23–0.36 mm wide, 2–3 × stem width, margins entire, apex truncate to slightly emarginate, recurved, insertion line slightly curved, base slightly auriculate, cells similar to leaf cells, marginal cells often ± isodiametric, 8–15 × 5–12 µm, median cells elongate, 20–25 × 5–12(–18) µm.

Systematic position

The specimens were assigned to the subfamily Ptychanthoideae of the Lejeuneaceae. The assignment to Lejeuneaceae was based on the complicate bilobed leaves (the leaves are divided into lobe and lobule, with the ventral lobule folded against the dorsal lobe), the incubous insertion of the leaves, and the presence of underleaves. The assignment to the Ptychanthoideae was possible due to two further characters, the broad ventral merophytes consisting of approximately five cell rows and the entire, undivided, emarginate underleaves. Extant Lejeuneaceae are common tropical epiphytes. Representatives of this family also dominate the liverwort floras of the Miocene Dominican and Mexican amber forests (Gradstein 1993; Reiner-Drehwald et al. 2012; Heinrichs et al. 2014a).

The two investigated specimens lack generative structures such as perianths and female bracts. Furthermore the structure of the lobule is unclear. Without these characters, conclusive assignment to a specific genus is not possible. However, the specimens share the same vegetative morphology and likely represent a single species of the pantropical genus *Mastigolejeunea*. The main characters that support this hypothesis are the rounded lobe apex, the oblique free lobule margin continuing into the ventral margin of the leaf lobe, and the elongate leaf cells. The shape of the leaf cells is difficult to discern because the majority of leaf cells appear collapsed. Nevertheless, several clearly uncollapsed leaf cells are elongate (Figs 1c, d; 2c, d). Moreover, the majority of underleaf cells are not collapsed and clearly elongate (Figs 1e; 2c).

Alternative genera to which the fossils could be assigned are a less likely match than *Mastigolejeunea*. *Thysananthus*, the sister genus of *Mastigolejeunea*, can only be separated by characters of the perianth

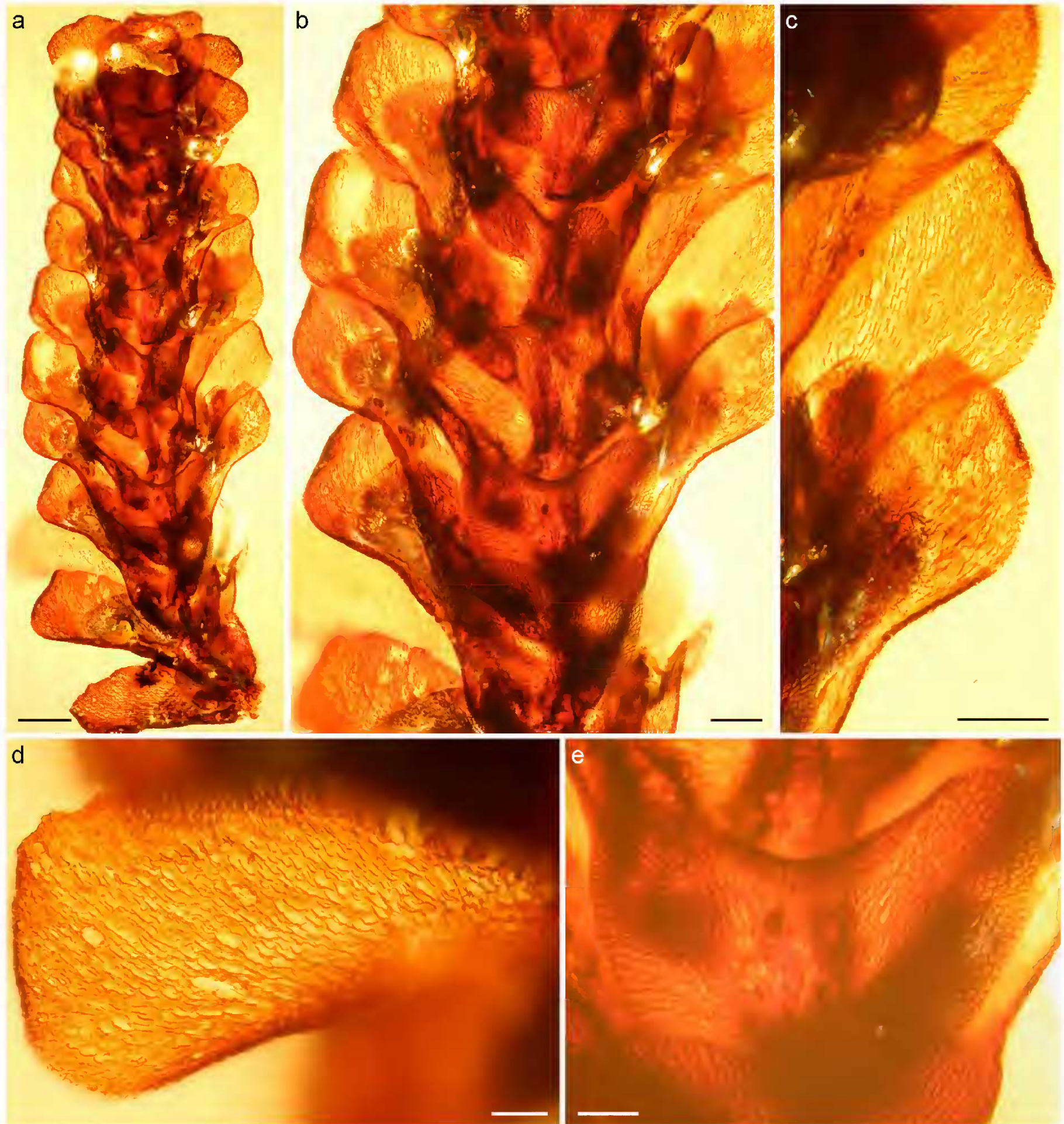


Fig. 1. Ptychanthoid Lejeuneaceae in Miocene Mexican amber (SMNS Mx 357), ventral view. **a**, sterile shoot; **b**, *ibid.*, close-up; **c,d** leaf lobes showing elongate leaf cells; **e**, underleaf. Scale bars 200 µm (a), 100 µm (b, c); 50 µm (d, e).

(Sukkharak and Gradstein, 2014). However, *Thysananthus* has its centre of diversity in Southeast Asia and the extant Neotropical species *Thysananthus amazonicus* (Spruce) Schiffn. is provided with acute leaf lobes (Gradstein 1994). *Schiffneriolejeunea* can also not be excluded based on the observed character states but has its centre of diversity in the Palaeotropics. *Caudalejeunea* is not known to have recurved underleaves. Finally, the fossils also share some characters with *Omphalanthus* (Lejeuneoideae), but this genus is characterized by its green to yellowish-brown colour, more or less adpressed, rounded to elongate rather than emarginate underleaves and (sub)isodiametric leaf cells (Gradstein et al. 2001). The fossils on the other hand are dark brown with somewhat spreading, emarginate underleaves and elongate leaf cells. Thus, based on the incomplete morphological evidence available, we hypothesize that the fossils belong to the genus *Mastigolejeunea*.

So far, two fossil species of *Mastigolejeunea* have been described, *Mastigolejeunea bidentula* Gradst. from Miocene Dominican amber and *Mastigolejeunea contorta* (Göpp. and Berendt) Gradst. & Grolle from Eocene Baltic amber (Grolle et al. 2004). Both taxa differ from the Mexican inclusions by the less emarginate and

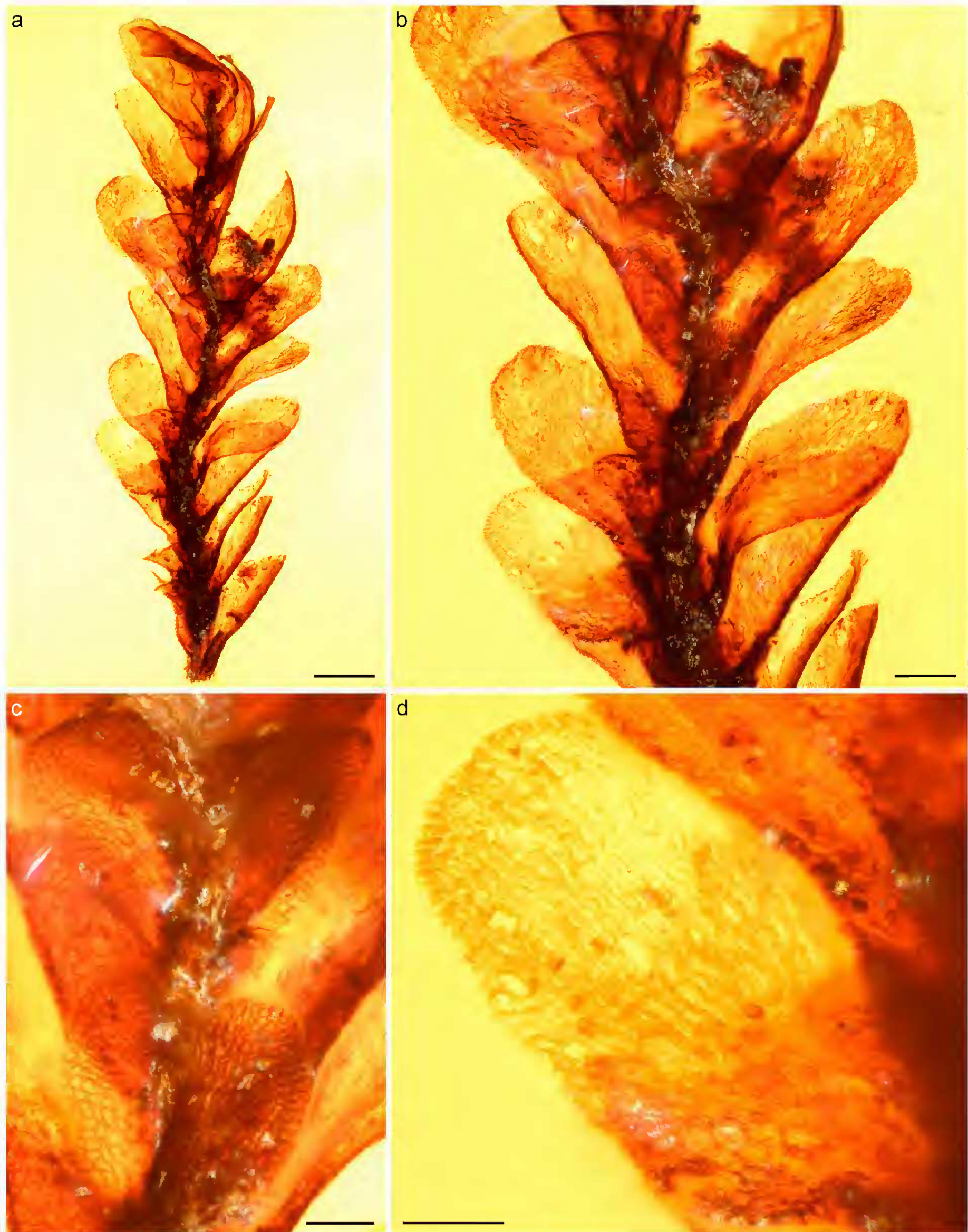


Fig. 2. Ptychanthoid Lejeuneaceae in Miocene Mexican amber (SMNS Mx 352), ventral view. **a**, sterile shoot; **b**, *ibid.*, close-up; **c**, underleaves; **d**, leaf lobe showing elongate leaf cells. Scale bars 200 µm (a), 100 µm (b); 50 µm (c,d).

at best weakly recurved underleaves. Further Dominican amber fossils have been assigned to the extant *Mastigolejeunea auriculata* (Wils. & Hook.) Steph. (Gradstein, 1993), and we cannot reject relationships of the incompletely preserved Mexican amber fossils to this taxon. However, more recent research has shown homoplastic characters and polyphyly within the Ptychanthoideae, and *Mastigolejeunea auriculata* was shown to include two independent entities (Sukkharak et al. 2011). Indeed, species of epiphytic liverworts may in fact represent recently emerged complexes of morphologically cryptic or semicryptic local endemics (Ramaiya et al. 2010). Therefore, until a comprehensive dated phylogeny of *Mastigolejeunea* is available, assignments within the genus may be tenuous. The Mexican specimens thus cannot be assigned a systematic position within *Mastigolejeunea*.

Perspectives

The insect fauna found in Mexican amber resembles that in Dominican amber (Solórzano Kraemer 2007), and we can expect this pattern to hold true for the bryoflora. The four liverwort fossils available at present suggest this similarity at generic level and confirm the frequent presence of Lejeuneaceae in the Miocene *Hymenaea* forests of the Caribbean and adjacent areas. The Mexican and Dominican amber inclusions are currently the only available tropical Cenozoic bryophyte fossils (Frahm and Newton 2005). The scarcity of bryophyte fossils, however, makes a more accurate reconstruction of the bryoflora of the Miocene Mexican amber forest difficult.

It is probable that the lack of bryophytes identified from amber deposits is either due to the low fossilization rate of these delicate organisms or due to the fact that most amber collectors focus on arthropods rather than on inclusions of plants. The latter scenario seems more likely. For example, no bryophytes have been described from the entomologically rich amber deposit of Oise discovered in 1996 (Nel et al. 1999), despite the apparent abundance of botanical inclusions (Nel et al. 2013). Moreover, the relatively newly discovered Eocene Indian amber deposits from the Cambay basin (Mukherjee et al. 2005) have provided a vast arthropod fauna with some connections to Mexican and Dominican amber (Rust et al. 2010) but have yet to yield any descriptions of bryological fossils. This is also true for better established amber deposits such as those in Spain (Peñalver et al. 2010). In summary, there is likely a plethora of undiscovered botanical diversity in the known amber deposits.

We thus hope that this study will encourage researchers to direct their attention to botanical inclusions, particularly in Mexican amber. Further fossils will shed light on evolutionary rates and phylogeography. Finally, with a more complete Mexican amber fossil record, we expect a deeper understanding of the Mexican and Dominican Miocene palaeoecosystems.

Acknowledgments

We thank Günter Bechly (Stuttgart State Museum of Natural History) for a loan of amber specimens SMNS Mx 352 and Mx 357 and Andrew Rozefelds (Queensland Museum, South Brisbane) for comments on the botanical inclusions preserved in Australian Cape York amber. We dedicate this paper to our colleague Elizabeth A Brown (1956–2013).

References

- Cooper ED, Henwood MJ, Brown EA (2012) Are the liverworts really that old? Cretaceous origins and Cenozoic diversifications in Lepidoziaceae reflect a recurrent theme in liverwort evolution. *Biological Journal of the Linnean Society* 107: 425–441. <http://dx.doi.org/10.1111/j.1095-8312.2012.01946.x>
- Donoghue PCJ, Benton MJ (2007) Rocks and clocks: calibrating the Tree of Life using fossils and molecules. *Trends in Ecology and Evolution* 22: 424–31. <http://dx.doi.org/10.1016/j.tree.2007.05.005>
- Feldberg K, Heinrichs J, Schmidt AR, Váňa J, Schneider H (2013) Exploring the impact of fossil constraints on the divergence time estimates of derived liverworts. *Plant Systematics and Evolution* 299: 585–601. <http://dx.doi.org/10.1007/s00606-012-0745-y>
- Feldberg K, Schneider H, Stadler T, Schäfer-Verwimp A, Heinrichs J (2014) Epiphytic leafy liverworts diversified in angiosperm-dominated forests. *Scientific Reports* 4: 5974. <http://dx.doi.org/10.1038/srep05974>
- Frahm JP (2004) A new contribution to the moss flora of Baltic and Saxon amber. *Review of Palaeobotany and Palynology* 129(1): 81–101. <http://dx.doi.org/10.1016/j.revpalbo.2003.11.004>
- Frahm JP (2010) *Die Laubmoosflora des baltischen Bernsteinwaldes*. (Weissdorn: Jena)
- Frahm JP, Newton A (2005) A new contribution to the moss flora of Dominican amber. *The Bryologist* 108: 526–536. [http://dx.doi.org/10.1639/0007-2745\(2005\)108\[0526:ANCTTM\]2.0.CO;2](http://dx.doi.org/10.1639/0007-2745(2005)108[0526:ANCTTM]2.0.CO;2)
- Gradstein SR (1993) New fossil Hepaticae preserved in amber of the Dominican Republic. *Nova Hedwigia* 57: 353–374.

- Gradstein SR (1994) Lejeuneaceae: Ptychantheae, Brachiolejeuneae. *Flora Neotropica Monograph* 62: 1–216.
- Gradstein SR, Churchill SP, Salazar-Allen N (2001) Guide to the bryophytes of tropical America. *Memoirs of the New York Botanical Garden* 86: 1–577.
- Grimaldi DA (1996) *Amber: window to the past*. (Harry N. Abrams, New York)
- Grolle R (1984) *Lejeunea palaeomexicana* n. sp., das erste Moos aus Mexikanischem Bernstein. *Stuttgarter Beiträge zur Naturkunde, Serie B*, 108: 1–7.
- Grolle R, Meister K (2004) *The liverworts in Baltic and Bitterfeld amber*. (Weissdorn, Jena)
- Grolle R, Meister K, So ML (2004) New evidence on the taxonomic position of the Eocene *Jungermanniites contortus* (Jungermanniales: Lejeuneaceae): *Cryptogamie, Bryologie* 25: 117–123.
- Hand S, Archer M, Bickel D, Creaser P, Dettmann M, Godthelp H, Jones A, Norris B, Wicks D (2010) Australian Cape York amber. Pp. 69–79 In: Penney D (ed.) *Biodiversity of fossils in amber from the major world deposits*. (Siri Scientific Press, Manchester)
- Hedenäs L, Heinrichs J, Schmidt AR (2014) Bryophytes of the Burmese amber forest: Amending and expanding the circumscription of the Cretaceous moss genus *Vetiplanaxis*. *Review of Palaeobotany and Palynology* 209: 1–10. <http://dx.doi.org/10.1016/j.revpalbo.2014.05.008>
- Heinrichs J, Hentschel J, Wilson R, Feldberg K, Schneider H (2007) Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. *Taxon* 56: 31–44.
- Heinrichs J, Reiner-Drehwald ME, Feldberg K, Grimaldi DA, Nascimbene PC, von Konrat M, Schmidt AR (2011) *Kaolakia borealis* nov. gen. et sp. (Porellales, Jungermanniopsida): A leafy liverwort from the Cretaceous of Alaska. *Review of Palaeobotany and Palynology* 165 (3): 235–240. <http://dx.doi.org/10.1016/j.revpalbo.2011.04.002>
- Heinrichs J, Reiner-Drehwald ME, Feldberg K, von Konrat M, Hentschel J, Váňa J, Nascimbene P, Grimaldi D, Schmidt AR (2012) The leafy liverwort *Frullania* (Jungermanniopsida) in the Cretaceous amber forest of Myanmar. *Review of Palaeobotany and Palynology* 169: 21–28. <http://dx.doi.org/10.1016/j.revpalbo.2011.10.002>
- Heinrichs J, Schäfer-Verwimp A, Boxberger J, Feldberg K, Solórzano Kraemer MM, Schmidt AR (2014a) A fossil species of *Ceratolejeunea* (Lejeuneaceae, Porellales) preserved in Miocene Mexican amber. *The Bryologist* 117: 10–14. <http://dx.doi.org/10.1639/0007-2745-117.1.010>
- Heinrichs J, Schäfer-Verwimp A, Feldberg K, Schmidt AR (2014b) The extant liverwort *Gackstroemia* (Lepidolaenaceae, Porellales) in Cretaceous amber from Myanmar. *Review of Palaeobotany and Palynology* 203: 48–52. <http://dx.doi.org/10.1016/j.revpalbo.2014.01.004>
- Heinrichs J, Schmidt AR (2010) An inclusion of *Frullania* subgen *Diastaloba* s.l. (Frullaniaceae, Porellales) in Dominican amber. *Tropical Bryology* 31: 91–94.
- Ignatov MS, Perkovsky EE (2011) Mosses from Rovno amber (Ukraine). *Arctoa* 20: 1–18. <http://dx.doi.org/10.15298/arctoa.20.01>
- Iturralde-Vinent M, MacPhee R (1996) Age and paleogeographical origin of Dominican amber. *Science* 273: 1850–1852. <http://dx.doi.org/10.1126/science.273.5283.1850>
- Konstantinova NA, Ignatov MS, Perkovsky EE (2012) Hepatics from Rovno amber (Ukraine). *Arctoa* 21: 265–271. <http://dx.doi.org/10.15298/arctoa.21.25>
- Laenen B, Shaw B, Schneider H, Goffinet B, Paradis E, Désamuré A, Heinrichs J, Villarreal JC, Gradstein SR, McDaniel SF, Long DG, Forrest LL, Hollingsworth ML, Crandall-Stotler B, Davis EC, Engel J, von Konrat M, Cooper ED, Patino J, Cox CJ, Vanderpoorten A, Shaw A (2014) Extant diversity of bryophytes emerged from successive post-Mesozoic diversification bursts. *Nature Communications* 5: 6134. <http://dx.doi.org/10.1038/ncomms6134>
- Langenheim JH (1995) Biology of amber-producing trees: Focus on case studies of *Hymenaea* and *Agathis*. Pp. 1–31 In: Anderson KB, Crelling JC (eds) *Amber, resinite, and fossil resins. ACS Symposium Series* 617. (American Chemical Society: Washington DC)
- Mukherjee D, Bhupathy S, Nixon A (2005) First record of an exceptionally diverse and well preserved amber-embedded biota from Lower Eocene (~ 52 Ma) lignites, Vastan, Gujarat. *Current Science* 89(8): 1328–1330.
- Nel A, de Ploëg G, Dejax J, Dutheil D, de Franceschi D, Gheerbrant E, Godinot M, Hervet S, Menier J-J, Augé M, Bignot G, Cavagnetto C, Duffaud S, Gaudant J, Hua S, Jossang A, de Lapparent de Broin F, Pozzi J-P, Paicheler J-C, Bouchet F, Rage J-C (1999) Un gisement sparnacien exceptionnel à plantes, arthropodes et vertébrés (Éocène basal, MP7): Le Quesnoy (Oise, France) *Comptes-Rendus de l'Académie des Sciences* 329: 65–72.
- Nel P, Schmidt AR, Bässler C, Nel A (2013) Fossil thrips of the family Uzelothripidae suggest 53 million years of morphological and ecological stability. *Acta Palaeontologica Polonica* 58: 609–614.
- Peñalver E, Delclòs X, Penney D (2010) Spanish amber. Pp. 236–269 In: Penney D (ed.) *Biodiversity of fossils in amber from the major world deposits*. (Siri Scientific Press, Manchester)

- Poinar, GO Jr (1991) *Hymenaea protera* sp. n. (Leguminosae, Caesalpinioideae) from Dominican amber has African affinities. *Experientia* 47: 1075–1082. <http://dx.doi.org/10.1007/BF01923347>
- Poinar GO Jr, Brown AE (2002) *Hymenaea mexicana* sp. nov. (Leguminosae: Caesalpinioideae) from Mexican amber indicates Old World connections. *Botanical Journal of the Linnean Society* 139: 125–132. <http://dx.doi.org/10.1046/j.1095-8339.2002.00053.x>
- Ramaiya M, Johnson MG, Shaw B, Heinrichs J, Hentschel J, von Konrat M, Davison PG, Shaw AJ (2010) Morphologically cryptic biological species within the liverwort *Frullania asagrayana*. *American Journal of Botany* 97: 1707–1718. <http://dx.doi.org/10.3732/ajb.1000171>
- Reiner-Drehwald ME, Schmidt AR, Heinrichs J (2012) The genus *Lejeunea* in Miocene amber from the Dominican Republic. *Cryptogamie, Bryologie* 33: 33–38. <http://dx.doi.org/10.7872/cryb.v33.iss1.2012.033>
- Rust J, Singh H, Rana RS, McCann T, Singh L, Anderson K, Sarkar N, Nascimbene PC, Stebner F, Thomas JC, Solórzano Kraemer MM, Williams CJ, Engel MS, Sahni A, Grimaldi DA (2010) Biogeographic and evolutionary implications of a diverse paleobiota in amber from the early Eocene of India. *Proceedings of the National Academy of Sciences of the United States of America* 107: 18360–18365. <http://dx.doi.org/10.1073/pnas.1007407107>
- Schmidt AR, Perrichot V, Svojtka M, Anderson KB, Belet KH, Bussert R, Dörfelt H, Jancke S, Mohr B, Mohrmann E, Nascimbene PC, Nel A, Nel P, Ragazzi E, Roghi G, Saupe EE, Schmidt K, Schneider H, Selden PA, Vávra N (2010) Cretaceous African life captured in amber. *Proceedings of the National Academy of Sciences of the United States of America* 107: 7329–7334. <http://dx.doi.org/10.1073/pnas.1000948107>
- Schmidt AR, Ragazzi E, Coppelotti O, Roghi G (2006) A microworld in Triassic amber. *Nature* 444: 835. <http://dx.doi.org/10.1038/444835a>
- Skalski AW, Veggiani A (1990) Fossil resin in Sicily and the Northern Apennines: geology and organic content. *Prace Muzeum Ziemi* 41: 37–49.
- Solórzano Kraemer MM (2007) Systematics, palaeoecology, and palaeobiogeography of the insect fauna from Mexican amber. *Palaeontographica Abteilung A* 282: 1–133.
- Solórzano Kraemer MM (2010) Mexican amber. Pp. 42–56 In: Penney D (ed.) *Biodiversity of fossils in amber from the major world deposits*. (Siri Scientific Press, Manchester)
- Sukkharak P, Gradstein SR (2014) A taxonomic revision of the genus *Mastigolejeunea* (Marchantiophyta: Lejeuneaceae). *Nova Hedwigia*, online early: DOI: <http://dx.doi.org/10.1127/0029-5035/2014/0206>
- Sukkharak P, Gradstein SR, Stech M (2011) Phylogeny, taxon circumscriptions, and character evolution in the core Ptychanthoideae (Lejeuneaceae, Marchantiophyta). *Taxon* 60: 1607–1622.
- Sun Y, He X, Glenn D (2014) Transantarctic disjunctions in Schistochilaceae (Marchantiophyta) explained by early extinction events, post-Gondwanan radiations and palaeoclimatic changes. *Molecular Phylogenetics and Evolution* 76: 189–201. <http://dx.doi.org/10.1016/j.ympev.2014.03.018>